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Molecular diagnostics of families with long-QT syndrome

Ewa Moric-Janiszewska, Marta Głowacka

Department of Biochemistry, Medical University of Silesia, Sosnowiec, Poland

Abstract

The Department of Pediatric Cardiology, Medical University of Silesia in Katowice-Ligota, studied 24 patients with clinically diagnosed (using ECG) long-QT syndrome (LQTS) in 18 cases. Nine patients were diagnosed with LQT1 and nine with LQT2. The other six individuals were healthy, with no symptoms characteristic for prolonged QT syndrome, but came from families with confirmed disease occurrence. The study was conducted on members of four families. In order to search for mutations (using mSSCP and sequencing), genomic DNA was obtained from patients to determine the expression levels of the genes KCNQ1 *and* KCNH2 *(HERG), involved in the occurrence of clinical signs of disease. Total RNA was extracted from peripheral blood. Consent to the use of blood samples of patients had been given by the Bioethics Commission of the Medical University of Silesia.*

mSSCP analysis and sequencing did not confirm the occurrence of mutations in KCNQ1 *and* HERG *associated with the occurrence of LQTS. Analysis of gene expression profile of* KCNQ1 *and* HERG *confirmed the presence of disease in people with a known clinical diagnosis. Overexpression, as well as reduced expression, was observed for the examined genes.* KCNQ1 *was inhibited in two families, whereas* HERG *was reduced in one and overexpressed in the other. Gene expression profile analysis showed abnormal expressions of* KCNQ1 *and* HERG *in healthy subjects, which may be a sign of predisposition to develop the disease. The novelty of our study involved the use of total mRNA isolated from human peripheral blood, and the very limited evidence in the literature to date regarding the assessment of gene expression profile of* HERG *and* KCNQ1 *in relation to the presence of prolonged QT syndrome.* (Cardiol J 2012; 19, 2: 159–167)

Key words: long-QT syndrome, molecular diagnostics, QRT-PCR (real-time PCR), mSSCP analysis, sequence analysis

Introduction

Congenital prolonged QT syndrome is a rare genetically determined disease associated with impaired function or structure of ion channels located in the heart. This results in abnormal cardiac repolarization and the risk of the appearance of cardiac arrhythmias and sudden cardiac death (SCD). Characteristics are: prolonged QT interval in the ECG, the incidence of syncope, and sudden cardiac arrest [1, 2]. To date, 12 genes have been discovered, mutations of which are responsible for long QT syndrome (LQTS). All candidate genes are located on autosomes, which explains the lack of disease association with sex. The genes, mutations of which cause LQTS1 i.e. KvLQT1, KCNQ1, and LQTS2 i.e. HERG-KNNH2, encode proteins of potassium channels, while LQTS3 (SCN5A) is caused by mutations in the gene encoding the sodium channel protein [3, 4].

Address for correspondence: Ewa Moric-Janiszewska, PhD, Department of Biochemistry, Medical University of Silesia, ul. Narcyzów 1, 41–200 Sosnowiec, Poland, tel: +48 32 364 10 06, e-mail: ejaniszewska@sum.edu.pl

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The primary goal of treatment is to prevent the LQTS episodes of arrhythmias and sudden cardiac arrest. Beta-blockers are commonly used as drugs of choice for all patients with LQTS, but the introduction of therapy, depending on the patient's genotype, has proved to be useful in the treatment of various types of LQTS. In LQTS1, nikorandil is applied, which shortens the QT interval. In LQTS2, due to the large loss of potassium, spironolactone and potassium salts are used. In LQTS3, antiarrhythmic drugs such as lidocaine, mexiletine, and flecainide have proved effective [1, 3, 5]. In situations where treatment with β-blockers appears to be ineffective, LQTS3 patients are recommended for cardioverter-defibrillator implantation [6].

There have been reports that 50–60% of patients with LQTS have the LQTS1 genotype, 35––40% the LQTS2 genotype, and 8% the LQTS3 genotype. Mutations in LQTS1 and LQTS2 are associated with an earlier manifestation of symptoms and a low risk of sudden death, whereas LQTS3 mutations are connected with the later appearance of symptoms and a higher risk of SCD [7]. Due to the frequent absence of clinical symptoms in up to 40% of carriers of mutant genes, which do not always verify the clinical diagnostic criteria, these patients should be diagnosed using molecular methods.

The early identification of LQTS is important because of the high mortality in untreated patients with symptoms (20% of patients die within one year of the first loss of consciousness, and 50% of patients have died ten years after such an episode) [3, 7]. Carriers of mutations in whom the disease has not been revealed have a 10% risk of arrhythmia before the age of 40, especially when additionally taking QT prolonging drugs or when they are exposed to factors that initiate cardiac arrhythmias [8]. In asymptomatic carriers of mutations, genotypic identification has some limitations because not all mutations that cause LQTS are recognizable in a genotype [7].

Molecular analysis

Clinical characteristics of patients

In our study, we called the four families: family 1, family 2, family 3 and family 4.

Among the eight members of family 1, of three different generations, only one person was not studied. After performing an electrocardiogram (ECG), it turned out that all the women in this family suffered from LQTS1. Clinical examination also confirmed the presence of LQTS1 in a male child of a sick mother and a healthy father.

The entire two-generation family 2 underwent study (four persons). LQTS1 was diagnosed by ECG in the father and his two children, but the mother was healthy.

The four-generation 11–member family 3 was diagnosed by ECG: LQTS2 symptoms were revealed in four patients, and two patients from the first generation were not analyzed. Two sisters from the second generation were healthy. Two of the three children of one of these sisters were sick, but their biological father did not suffer from LQTS2. In the last studied generation, the disease was present in all descendants of a sick mother and a healthy father.

In the three-generation family 4, of six individuals, five were examined. All patients from family 4 were diagnosed with LQTS2.

The pedigrees of the four families are shown in Figure 1.

The study was approved by the local bioethical committee and all patients gave their informed consent.

Qualitative assessment of total RNA extracts

In order to analyze the quality, DNA and RNA extracts were analyzed using the technique of electrophoresis in 1% agarose gel stained with ethidium bromide.

Quantitative assessment of DNA and RNA extracts

Genomic DNA was obtained from 5 mL of whole blood using proteinase K and universal lysis buffer, while total RNA (from 1 mL of whole blood) was extracted using phenosol, chloroform and isopropanol. Quantitative assessment was made using a calculator GeneQuant Pro (Amersham Biosiences). The absorbance of samples was measured at wavelengths of 260 nm and 280 nm. Absorbance at 260 nm was associated with a concentration of DNA and RNA in the samples. Furthermore, the purification factor (ratio of absorbance A280nm/A260nm) was detemined. The highest concentration of RNA was observed in patient number 30. It was 0.428 μg/μL. Patient number 30 belonged to a group of individuals diagnosed with LQTS. The lowest concentration of RNA was observed in patient number 105: 0.040 μg/μL. This patient also was diagnosed with the disease. The average value of RNA concentration was 0.110 $\pm$ 0.082 μg/μL. The purity factor averaged 1.544 $\pm$ 0.160. The maximum value of the extract concentration of RNA in patients diagnosed clinically was 1.428 μg/μL, and the minimum was 0.040 μg/μL. The average concentration was 0.111 $\pm$ $\pm$ 0.091 μg/μL, while the purification average rate

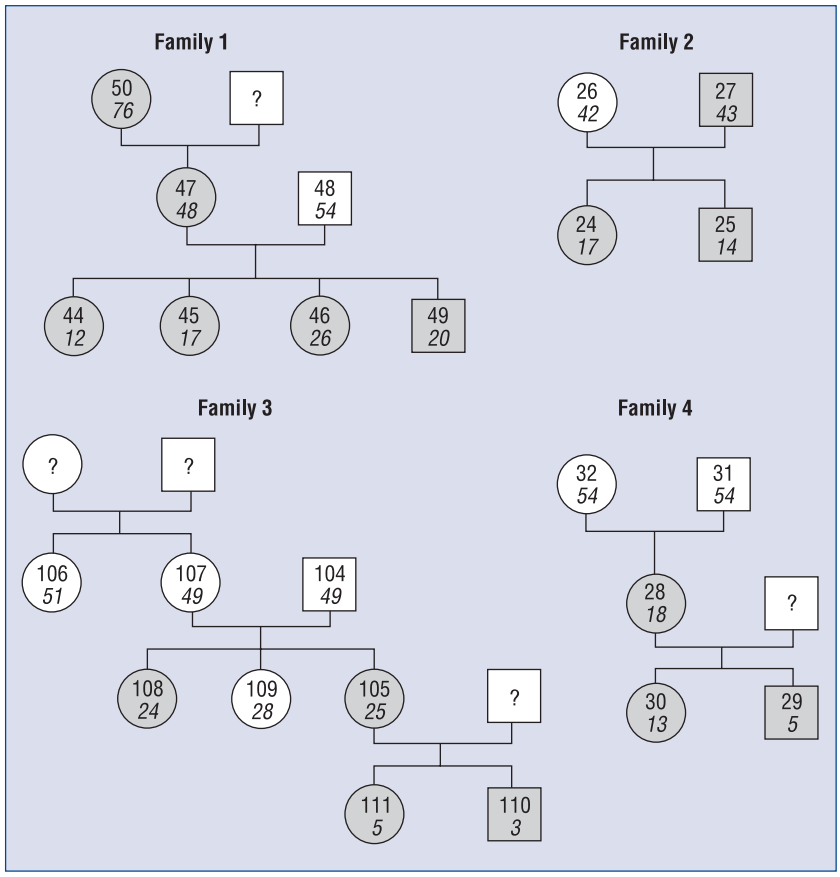

Figure 1. Pedigrees of surveyed families. Circles indicate females, squares indicate males. Sample number shown in normal font. Age of family members shown in italics. Grey color symbolizes the disease, while white stands for healthy person. Question mark means that the person was not subjected to the test.

was 1.528 ± 0.095. The value of the maximum concentration of RNA in the group of healthy subjects was 0.202 μg/μL, and the minimum was 0.064 μg/μL. The average concentration of total RNA was 0.105 ± 0.051 μg/μL, and was 0.006 μg/μL lower than in the diagnosed patients. The average purity ratio was 1.590 ± 0.234 and was 0.061 higher than in the group diagnosed with LQTS. The highest concentration of DNA was observed in patient number 30, who was diagnosed with the disease. This was 0.115 μg/μL. The lowest concentration of DNA was recorded in patient number 45 and was 0.018 μg/μL. This patient belonged to a group of people diagnosed with LQTS. The average value of concentration was 0.042 ± 0.022 μg/μL. The purity coefficient averaged 1.922 ± 0.338. The value of the maximum concentration of DNA extract in patients diagnosed clinically was 0.115 μg/μL, and the minimum was 0.018 μg/μL. The average concentration was 0.041 ± 0.024 μg/μL, while the purification average rate was 1.868 ± 0.230. The value of the maximum concentration of DNA in the group of healthy subjects was 0.074 μg/μL, and the minimum was 0.025 μg/μL. The average concentration of total DNA was 0.042 ± 0.020 μg/μL, and was 0.018 lower than in diagnosed patients. The average purity ratio was 2.083 ± 0.552 and was 0.214 higher than in the group diagnosed with LQTS.

Assessment of the size of PCR products

Extracts were subjected to genomic DNA amplification. Using polyacrylamide gel electrophoresis, the sizes of amplification reaction products were assessed. Polyacrylamide gel was then stained and analyzed by gel documentation system BASSYS1D (Biotec Fischer) and GelScan v.1.45 (Kucharczyk). The size of each product was evaluated by comparing its electrophoretic mobility and the mobility of restriction fragment pattern of plasmid pBR322 digested with HaeIII endonuclease. Statistical analysis was performed for all PCR products in order to verify the assumptions of equal value and the average band to determine the error in the calculation of the gel documentation system (results not shown). Amplified fragments of the studied genes were separated and submitted to polyacrylamide

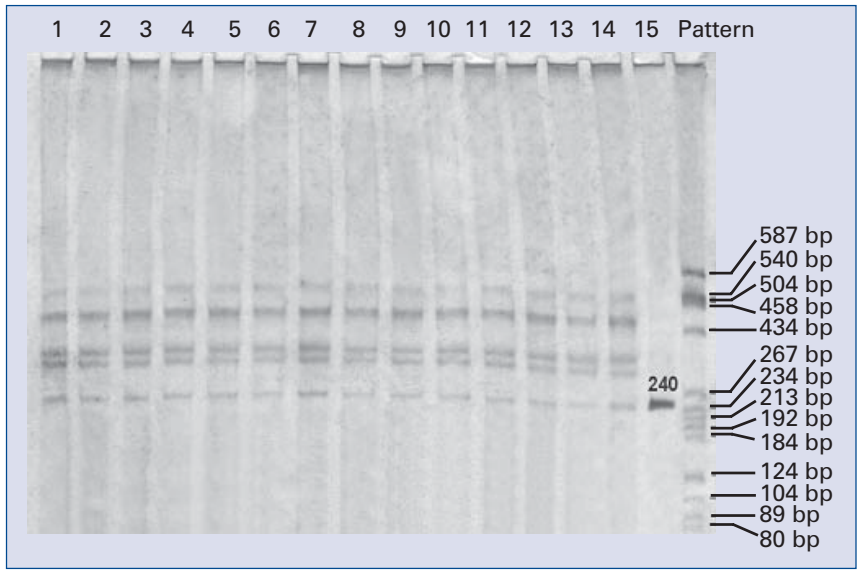

Figure 2. mSSCP analysis of *HERG* exon 3 (heterozygous). Tracks 1–14: Bands 1–4 — ssDNA; Band 5 — dsDNA (PCR product size undenatured 240 bp). Track 15: Native PCR product. Track 16: Pattern (plasmid pBR322 digested with HaeIII endonuclease).

gel. The specificity of the reaction was confirmed on densitometric analysis of PCR products, on the basis of the position of bands, also referred to the size of each amplification product (expressed in quantities of base pairs).

Analysis of *HERG* and *KCNQ1* genes using mSSCP technique

To establish the prevalence of mutations or polymorphisms in the studied patients, both those with LQTS and those who were healthy, mSSCP analysis of individual exons of *KCNQ1* and *HERG* genes was performed according to the procedure described previously. The figures in this study show the polyacrylamide gels after mSSCP analysis of *HERG* and *KCNQ1* genes with clearly visible bands, which illustrates the correct expression of the denatured samples and appropriately selected conditions of electrophoretic separation.

Figure 2 shows the results of mSSCP analysis of exon 3 of *HERG* gene for 14 patients. Paths 1–14 contain dsDNA and ssDNA fragments of *HERG* gene. dsDNA, which migrates faster, is visible at the bottom of the gel (240 bp), whereas the ssDNA fragments are located above. The fact that all the ssDNA bands are located at the same height in all cases shows the lack of mutations or polymorphisms in the material. Path 15 contains control native *HERG* gene PCR product (negative control), and track 16 contains the size marker (pBR322/ /HaeIII) (Fig. 2). mSSCP analysis of exon 3 of *HERG* gene identified the following location of ssDNA bands: 504 bp + 439 bp + 382 bp + 327 bp. The presence of four bands in this case indicates the heterozygosity (Fig. 2).

Figure 3 shows the results of mSSCP analysis of exon 6 of *KCNQ1* gene for 14 patients. In each track (1–15) there was non-denaturated PCR product (negative control) and in the last track (16) there was a size marker (pBR322/HaeIII). Gel obtained for *KCNQ1* gene exon 6 revealed the presence of two ssDNA bands, their position was as follows: 599 bp + + 448 bp. The presence of two bands indicates homozygosity of the test sequence. The fact that all the ssDNA bands are located at the same height in all cases is associated with a lack of mutations or polymorphisms in the material (Fig. 3).

Sequencing analysis of genes *KCNQ1* and *HERG*

In order to exclude or confirm the presence of mutations or polymorphisms in the patients studied, sequencing analysis was performed according to the previously described procedure. This analysis aimed to identify mutations (mSSCP, sequencing) which had not been confirmed as present in the families enrolled in the study. In the analysis of nucleotide sequence, all the genes tested were identical to gene sequences obtained from genetic databases, suggesting a lack of mutations in the studied genes (ENSG00000053918 and ENSG00000055118).

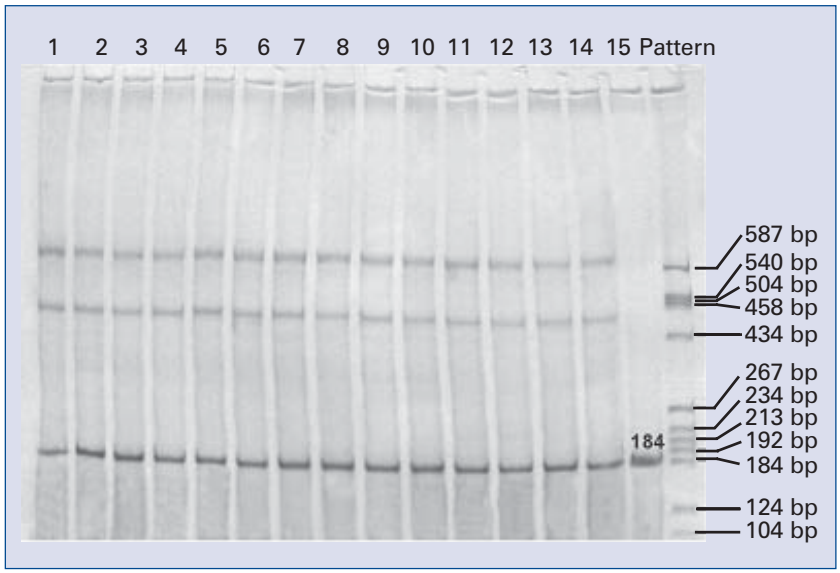

Figure 3. mSSCP analysis of *KCNQ1* exon 6 (homozygous). Tracks 1–14: Bands 1–2 — ssDNA; band 3 — dsDNA (PCR product size undenatured 240 bp). Track 15: Native PCR product. Track 16: Pattern (plasmid pBR322 digested with HaeIII endonuclease).

Analysis of the expression of genes *KCNQ1* and *HERG*

We analyzed two genes responsible for the occurrence of LQTS1 and LQTS2. We proved both overexpression and reduced expression of the genes examined. For the families surveyed, five values were calculated: the median, lower and upper quartile, the mean, and standard deviation. The results are shown in Table 1. The average value of the number of copies of *KCNQ1* mRNA in family 1 was 9,364 ± 22,789.5. People diagnosed with LQTS1 presented reduced expression of *KCNQ1*. A healthy person also had a smaller number of copies of *KCNQ1* mRNA. For family 2, the gene copy number ranged from 214 to 1,082. In patients with clinically confirmed disease, there was a significant reduction in *KCNQ1* gene expression. The average value of the test gene copy number was 620 ± ± 396.8.

In family 3, the highest value of *HERG* gene expression (2,146,723 copies) was present in a patient diagnosed with LQTS. In all patients suffering from LQTS2, we clearly observed increased expression of the gene (in one patient only slightly elevated). The average value of *KCNE2* gene copy number was 651,948 ± 941,692.

All members of family 4 had a reduced expression of *HERG*, but their values were different, which may be associated with symptoms in individual patients. Gene occurred in this family with an average number of copies of 1,330 ± 2,763.6.

Table 1. Descriptive statistics of families 1–4 (both sick and healthy taken into account).

Parameter	KCNQ1	β-actin
Family 1		
Median	726	3,427
Lower quartile	429.0	2,355.2
Upper quartile	1,414.9	660,715.6
Mean deviation	9,364	605,940.0
Standard deviation	22,789.5	1,125,933.0
Family 2		
Median	593.0	43,277.0
Lower quartile	336.6	24,056.6
Upper quartile	876.0	101,195.0
Mean deviation	620.0	81,975.0
Standard deviation	396.8	106,968.7
Family 3		
Median	204,965.0	8,913.0
Lower quartile	104,441.1	3,948.0
Upper quartile	717,817.3	11,151.0
Mean deviation	651,948.0	12,595.0
Standard deviation	941,692.0	15,710.3
Family 4		
Median	94.0	75,687.0
Lower quartile	2,763.7	51,821.0
Upper quartile	224.3	150,172.8
Mean deviation	1,330.0	160,476.0
Standard deviation	2,763.7	210,091.2

Discussion

Four families were tested, totaling 24 people: nine patients with LQTS1, nine with LQTS2, and six healthy. The family members were patients of the Pediatric Cardiology Medical University of Silesia in Katowice-Ligota. From the peripheral blood, total RNA and genomic DNA were isolated. The average value of the total RNA concentration was 0.110 ± 0.082 μg/μL, with a coefficient purity of 1.544 ± 0.160, which was lower than the value described in the literature [9]. This may result from phenol contamination of samples or insufficient drying time, or storing blood for too long. The average value of the concentration of DNA extracts was 0.042 ± 0.022 μg/μL, with a coefficient purity of 1.922 ± 0.338, which was higher than described in the literature [10]. This may indicate contamination as with the RNA. Genomic DNA was used to amplify exons 1–6 of *KCNQ1* and 1–15 of *HERG* gene. We present the results of analysis for exons 2–7 of *KCNQ1* gene, encoding α subunit of the slowly activating potassium channel and exons 6–9 of *HERG* encoding α subunit of the rapidly activating potassium channel.

We analyzed exons using two techniques: mSSCP and sequencing method. This paper presents the results for the *HERG* gene exon 3 and exon 6 of gene *KCNQ1*. Literature data indicates that these methods are useful in detecting polymorphisms and mutations in genes associated with the occurrence of LQTS [2, 12–18].

In this work, we used a variation of the SSCP technique known as mSSCP (multitemperature SSCP) due to its simplicity, greater sensitivity, reduced cost and time analysis. mSSCP analysis of *HERG* and *KCNQ1* revealed no mutations in these genes. We showed heterozygosity of *HERG* gene exon 3, visible as a pattern composed of four bands that are the equivalent of ssDNA, which indicates acceptable polymorphism in the nature rather than a mutation. Analysis of exon 6 of gene *KCNQ1* indicated its homozygotic sequence (two bands ssDNA). Other investigators from Medical University of Silesia (MUS) have described similar results [2]. By analyzing six exons of the gene *KCNQ1* they gained a wider spectrum of fringe patterns, confirmed the homozygosity and heterozygosity and lack of sequence mutation. The size of tested DNA fragments has a huge impact on the analysis sensitivity. The best results are achieved with a size of 150 bp, where the detection of changes reaches more than 95%. Above 300 bp, detection falls below 80% [19]. In this paper, the length of ssDNA fragments was 250–350 bp, so the mSSCP analysis could not reveal conformational variants of ssDNA providing a possible mutation. An important parameter is the temperature of the electrophoresis, which should not exceed 20°C, because it has a negative effect on the efficiency of creating conformers. The use of simultaneous electrophoresis at different temperatures increases the sensitivity of the method [2, 19]. In this paper, we used temperatures of 4°C, 10°C, and 25°C. The heat may cause the formation of unstable conformers, which, during electrophoresis, pass from one form to another, which is evident in the form of fuzzy bands and streaks [19]. This effect was not observed in this work. Sequencing is a method common among researchers dealing with LQTS [2, 11, 12, 15, 20–24]. The result of sequencing confirmed the absence of mutations in the analyzed genes. However, the exclusion of mutations in studied patients may not be definitive, because they might be present in the beta subunits of studied genes, which were not analyzed.

RNA material after isolation and quantitative and qualitative assessment was used as a template for QRT-PCR reactions to evaluate the transcriptional activity of genes tested by the number of copies of genes per 1 μg of total RNA.

The study revealed overexpression, and reduction in expression, of genes in LQTS patients and in healthy subjects. In family 1, reduced expression of *KCNQ1* was detected. The average number of *KCNQ1* mRNA copies was 9,364 ± 22,789.52. For β-actin gene it was 605,940 ± 1,125,933.

Reduced copy number of *KCNQ1* gene was also observed in family 2. The average number of mRNA copies was 620 ± 396.8359951 (β-actin-81975 ± ± 106,968.7). *KCNQ1* gene encodes the protein transmembrane β subunit of slowly activating potassium channel (Iks). Insufficient copy number of *KCNQ1* mRNA can lead to decreased translation of protein building potassium channel or result in an incomplete canal (built only with β subunit), which may lead to impaired cardiac repolarization or the abnormal transport of ions between the epithelial cells in various tissues [25].

HERG overexpression was identified in family 3. The average value of its gene mRNA copy number was 651,948 ± 941,691.98, while the β-actin was 12,595 ± 15,710.28. Both clinically recognized and healthy patients showed an increased number of *HERG* mRNA copies. This suggests an increased predisposition to illness or possible mutation carrier. *HERG* gene expression product is a protein forming α subunit of rapid potassium channel (IKr), which is responsible for the final phase

of repolarization in cardiomyocytes and for the prevention of premature stimulation of myocardial infarction. Due to the PAS (Per-Arnt-Sim) conservative region presence in N-terminal fragment of protein, which is responsible for regulating the process of deactivation of the channel, *HERG* overexpression (too much protein-forming subunit α) can affect this process [26].

In family 4, where all members were diagnosed with LQTS2, we observed a reduced number of copies of the gene *HERG* mRNA. The average value of the mRNA copy number was 1,330 ± 2,763.691079 and was significantly lower than the number of copies of β-actin mRNA 160,476 ± 210,091.2. The relationship between the reduced number of *HERG* mRNA copies and IKr operation may be responsible for abnormal repolarization of myocardial cells, too fast depolarization and lack of inhibition of early induced arrhythmias [26]. Results showed a reduction in the *KCNQ1* expression in both analyzed families.

What may be surprising is a smaller number of copies of this gene also in healthy subjects. This raises a suspicion as to the increased predisposition of these individuals to the disease, but this hypothesis requires further study. In family 3, which revealed overexpression of the *HERG* gene, healthy subjects had a larger number of gene copies, which could be similarly interpreted.

Finley et al. [27] analyzed the expression levels of *KCNQ1* and *HERG* in horse cardiomyocytes. mRNA molecules were obtained from the ventricle and auricle cells of the heart. Different expression levels of the studied genes were proven. In addition, expression levels of these genes were compared in horse, rat and ferret. The outcome was similar in all species (detected as increased number of copies of *HERG* mRNA) [27]. In 2008, [28] University of Montreal researchers analyzed the level of expression and tissue distribution of *HERG* and *KCNQ1* transcripts. They used commercially available cell lines, including human embryonic kidney cells, heart muscle cells, prostate cancer cells and breast cancer cells. Using QRT-PCR reactions, they confirmed the high level of expression of genes *KCNQ1* and *HERG* in cells of the pancreas, heart and colon, whereas they found low levels in breast cancer cells and cardiac muscle cells. They observed a higher level of expression of these genes in the right ventricle and right atrium of the heart compared to the left ventricle and left atrium [28].

The most recent advances in research on *KCNQ1* and *HERG* expression were presented by researchers from MUS [29]. As one of the first they used the whole blood as a material for expression analysis. *KCNQ1* and *HERG* mRNA copy numbers were significantly lower in healthy women than in healthy men. Comparison of transcriptional activity of these genes studied in healthy adults and children revealed a significantly higher number of *KCNQ1* mRNA copies and a lower number of *HERG* mRNA copies in healthy adults [29]. Studies have shown extremely high levels of *KCNQ1* mRNA and lower levels of *HERG* mRNA in adult patients aged under 55 compared to the group aged over 55. These results allow the conclusion that the differences in the expression of these genes are influenced by the age and sex of respondents [29].

In subsequent studies, researchers from MUS [30] evaluated the effect of the expression level of *KCNQ1* and *HERG* on disease occurrence. There was no relationship between mRNA copy numbers of studied genes and QT interval in patients with LQTS1. A negative correlation was observed between the number of *KCNQ1* mRNA copies and QT interval in patients with LQTS2. The number of copies of *HERG* mRNA had no effect on QT interval in patients with LQTS2. The reason for this could be the very small number of both patients and healthy subjects in the study group and that the disease among young people could still be undisclosed. The study demonstrated that symptomatic LQTS1 patients have statistically a much higher number of *KCNQ1* mRNA copies than those who are asymptomatic (i.e. the healthy) [30].

In our work, there was no difference in expression between healthy and sick people, and therefore it cannot be compared. Both sick and healthy patients have a similar (reduced) expression of *KCNQ1* gene, which may result from the carrier of mutation or evidence of predisposition to disease. In LQTS2, researchers have found higher levels of *HERG* gene expression in symptomatic compared to asymptomatic patients, but this difference was not as significant as in the case of LQTS1. Both hypotheses have to be confirmed in the future on a larger number of patients [30]. Our work did not confirm differences in expression between healthy and sick people, and therefore it cannot be compared.

Gene expression analysis of *KCNQ1* and *HERG* is ongoing, and the full picture is not yet understood. Advances in molecular biology in the diagnosis of LQTS tend to consider other factors that may affect the phenotypic demonstration of disease [22]. Doubt on this subject surrounds the source from which the material is obtained for genetic analysis. It is suspected that myocardial tissue might be more appropriate than peripheral

blood to study the expression of genes responsible for LQTS. At present, studies are being conducted to assess the suitability of a peripheral blood test to evaluate the expression of these genes [22]. Based on the accumulated results, the impact of the level of expression of *KCNQ1* and *HERG* on the occurrence of LQTS cannot be clearly identified. To improve the results of the analysis, it would be worth considering the acquisition of genetic material from biopsies obtained from heart muscle cells.

Possible limitations of the current study result from the fact that changes in gene expression are controlled by complex, nonlinear interactions between proteins, DNA, RNA and various metabolites. Analysis of gene expression using the QRT-PCR technique allows for the determination of the level of mRNA expression (gene copy number/per the μg of protein), but this method can only suggest possible changes in the concentration of the protein or its function. The expression of protein (protein concentration) may not correlate with the expression of mRNA.

Despite the existence of a direct correlation between gene expression and function of proteins, further analysis, e.g. analysis of proteins, will be necessary to clarify the role of the level of *KCNQ1* and *KCNH2* expression in the etiology of LQTS. Another limitation is that the tested gene has to be transcribed at detectable levels in available tissue samples. Myocardial biopsy is not routinely performed in the diagnosis of LQTS. Unigene National Center for Biotechnology Information and Gene Expression Omnibus suggest that the expression of *KCNH2* and *KCNQ1* is limited to the heart muscle, and their mRNA transcripts cannot be obtained from lymphocyte cultures. Miller et al. [22] demonstrated that cardiac-specific mRNA could be detected in peripheral blood without additional purification steps or cell culture and used as starting material for the study of mutations of genes associated with heart disease.

It will be important to conduct studies to replicate our observations. The estimation of single nucleotide polymorphisms in genes associated with LQTS [31] may also be a point of interest. Tomás et al. [32] reported that the *NOS1AP* gene polymorphism, correlating with QT prolongation in a population, plays a role in modulating the phenotypic presentation in patients with LQTS.

Conclusions

1. mSSCP analysis results and sequencing did not confirm the occurrence of *KCNQ1* and *HERG* mutations in the studied group of people.
2. Analysis of gene expression profile of *KCNQ1* and *HERG* confirmed the presence of disease in people with a known clinical diagnosis.
3. Overexpression was observed, as well as reduced expression, of the examined genes. *KCNQ1* showed reduced expression in two families, whereas *HERG* expression was reduced in one family and elevated in the other.
4. Gene expression profile analysis showed abnormal expression of *KCNQ1* and *HERG* in healthy subjects. This may suggest a predisposition to develop the disease.
5. In order to obtain a more reliable picture of the impact of tested genes expression on the presence of LQTS1 or LQTS2, it would be advisable to increase the number of people in the study group.
6. The novel feature of our study was to use total mRNA isolated from human peripheral blood and a very limited literature knowledge regarding the assessment of gene expression profile of *HERG* and *KCNQ1* in relation to the presence of prolonged QT syndrome.

Conflict of interest: none declared

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